

Appl. No. : 09/351,631
Filed : June 21, 1999

- DB-
Amended
- a) identifying in a known sequence or designing an amino acid sequence which forms a helix-turn-helix structure;
- b) substituting individual residues in said amino acid sequence to achieve a sequence having the same distribution of positively charged residues and cysteine residues as the distribution found in a protein having a sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 3, and SEQ ID NO: 5;
- c) synthesizing chemically or expressing by recombinant DNA techniques in liquid culture an antimicrobial protein comprising said substituted amino acid sequence; and
- d) isolating said antimicrobial protein.

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Sub 1

17. **(Twice Amended)** The protein fragment of Claim 1, wherein said protein fragment is a polypeptide containing a relative cysteine and tyrosine or phenylalanine spacing of Z-2X-C-3X-C-(10-12)X-C-3X-C-3X-Z (SEQ ID NOS: 34-36) wherein X is any amino acid residue, and C is cysteine, and Z is tyrosine or phenylalanine.

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Sub 1

18. **(Twice Amended)** The protein fragment of Claim 1, wherein said relative cysteine spacing comprises C-2X-C-3X-C-(10-12)X-C-3X-C-2X-C (SEQ ID NOS: 1-33) wherein X is any amino acid residue, and C is cysteine.

19. **(Twice Amended)** An isolated or purified protein fragment having antimicrobial activity, wherein said protein fragment is selected from the group consisting of:

- residues 29 to 73 of SEQ ID NO: 1
- residues 74 to 116 of SEQ ID NO: 1
- residues 117 to 185 of SEQ ID NO: 1
- residues 186 to 248 of SEQ ID NO: 1
- residues 29 to 73 of SEQ ID NO: 3
- residues 74 to 116 of SEQ ID NO: 3
- residues 117 to 185 of SEQ ID NO: 3
- residues 186 to 248 of SEQ ID NO: 3
- residues 33 to 75 of SEQ ID NO: 5
- residues 76 to 144 of SEQ ID NO: 5
- residues 145 to 210 of SEQ ID NO: 5
- residues 34 to 80 of SEQ ID NO: 7
- residues 81 to 140 of SEQ ID NO: 7

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residues 33 to 79 of SEQ ID NO: 8
residues 80 to 119 of SEQ ID NO: 8
residues 120 to 161 of SEQ ID NO: 8
residues 32 to 91 of SEQ ID NO: 21
residues 25 to 84 of SEQ ID NO: 22
residues 29 to 94 of SEQ ID NO: 24; and
residues 31 to 85 of SEQ ID NO: 25.

06 20. (Twice Amended) The protein fragment of claim 1 which is truncated, wherein said truncated protein fragment retains the antimicrobial activity of the nontruncated protein fragment.

07 21. (Amended) A homologue of any of the protein fragments of claim 1.

08 09 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100
[Please cancel claims 22 and 23]

30. (Amended) A composition comprising a protein fragment according to claim 19 together with an agriculturally-acceptable carrier diluent or excipient.

31. (Amended) A composition comprising a protein fragment according to claim 19 together with a pharmaceutically-acceptable carrier diluent or excipient.

09 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64 65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95 96 97 98 99 100
34. (Twice Amended) A method of controlling microbial infestation of a plant, the method comprising treating said plant with an effective amount of the composition according to claim 11 for a period sufficient to inhibit microbial infestation of the plant.

10 39. (Amended) A method of controlling microbial infestation of a mammal, the method comprising treating the mammal with a composition according to claim 31.

11 42. (Amended) The method of claim 16, further comprising testing the antimicrobial protein for antimicrobial activity.

[Please add the following claims:]

012 43. A method of controlling microbial infestation of a plant, the method comprising treating said plant with an effective amount of the composition according to claim 30 for a period sufficient to inhibit microbial infestation of the plant.

013 44. A method of controlling fungal infestation of a plant by reducing the number of fungi, the method comprising treating said plant with an antimicrobial protein in an amount effective to reduce the number of said fungi, wherein:

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said antimicrobial protein comprises a polypeptide comprising a cysteine spacing of C-3X-C-(10-12)X-C-3X-C (SEQ ID NOS: 37-39) wherein X is any amino acid residue, and C is cysteine.

45. A method of controlling fungal infestation of a plant, the method comprising treating said plant with an effective amount of a composition comprising an antimicrobial protein together with an agriculturally-acceptable carrier diluent or excipient for a period sufficient to inhibit fungal infestation of the plant, wherein:

said antimicrobial protein comprises a polypeptide comprising a cysteine spacing of C-3X-C-(10-12)X-C-3X-C (SEQ ID NOS: 37-39) wherein X is any amino acid residue, and C is cysteine.

IN THE DRAWINGS

Please replace sheets 1 to 21 of the drawings with the accompanying sheets 1 to 21.

REMARKS

The claims have been amended to more precisely define the invention and in connection with objections taken by the Examiner. No new matter has been added by the amendments.

With regard to claim 1, the amendment to the formula merely limits the number of X residues between the two C-3X-C motifs to 11 or 12 which numbers were previously recited in the claim by virtue of the "(10-12)X" recitation. Claim 19 has been reworded as an independent claim but in the initial portion thereof has been amended to read in the same manner as claim 1 from which it formerly depended. The amendment of claim 34 merely comprises the substitution of "controlling" for "inhibition" thereby reverting to the claim as originally worded.

Claims 17, 18, 20, 21 and 42 have been amended to recite "claim 1" rather than "Claim 1" so that the claims as a whole have a uniform format. Claims 2, 16, 17 and 18 have had the format of the sequence listing identifiers amended to "SEQ ID NO: X" rather than "SEQ ID NO:X", again to introduce a uniform format throughout the claims.

The amendments to claims 30 and 31 have been made to correct the dependency thereof in view of the cancellation of the claims on which they formerly depended and to have the claims read consistently with the antecedent claims. The amendment of claim 39 is merely to correct the dependency of the claim.